JOHN COUNSELL, PHD

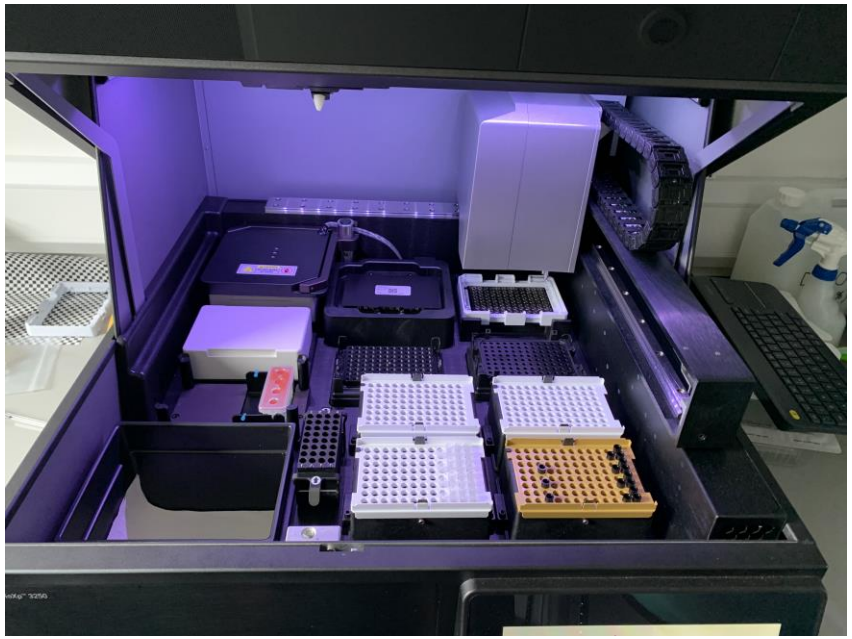
UCL DIVISION OF SURGERY AND INTERVENTIONAL SCIENCE

CENTRE FOR TARGETED INTERVENTION

Investigating Liver and Brain Gene Therapies for MSUD

Research Group

- New group, formed in 2022
- Based in Soho, London in the Division of Surgery and Interventional Science
- Team consists of 7 researchers developing new treatments for genetic diseases
- Projects cover different areas of medicine
- We specialise in designing and engineering therapies based on DNA
- Our mission is to engineer these therapies to better serve patients with incurable diseases



Strategic Position Within UCL

UCLH

Royal Free Hospital

Our Group

GOSH

Queen's Square Neurology

Research guided by conversations with medical doctors

What is gene therapy?

"*Utilising or controlling genetic material to treat or prevent disease*"

Can involve:

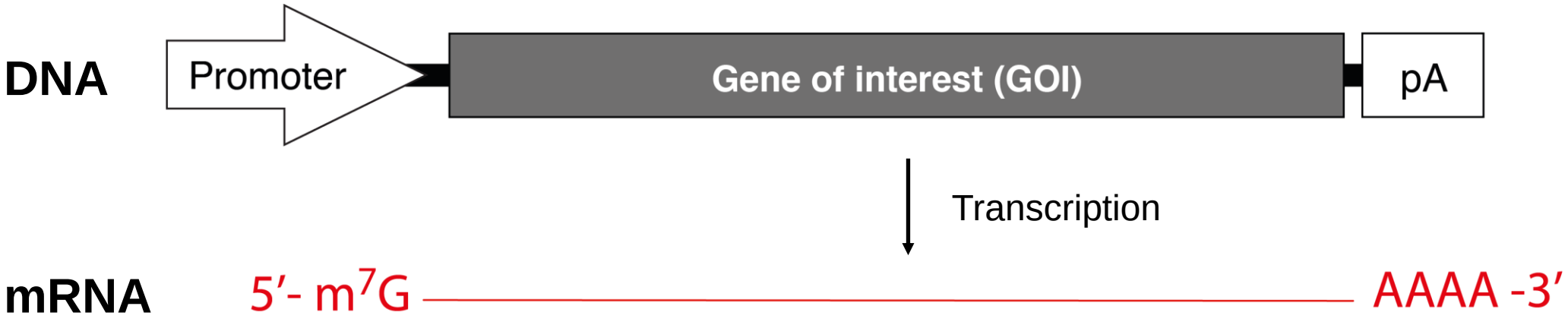
- ***Gene addition*** (add a gene when one is mutated)
- ***Gene silencing*** (suppress expression of a gene that causes disese)
- ***Gene editing*** (repair, replace, or control a gene in the patient's genome)

Can be delivered:

- ***In vivo*** (direct injection to the body)
- ***Ex vivo*** (treat patient cells outside of the body, before re-transplanting)

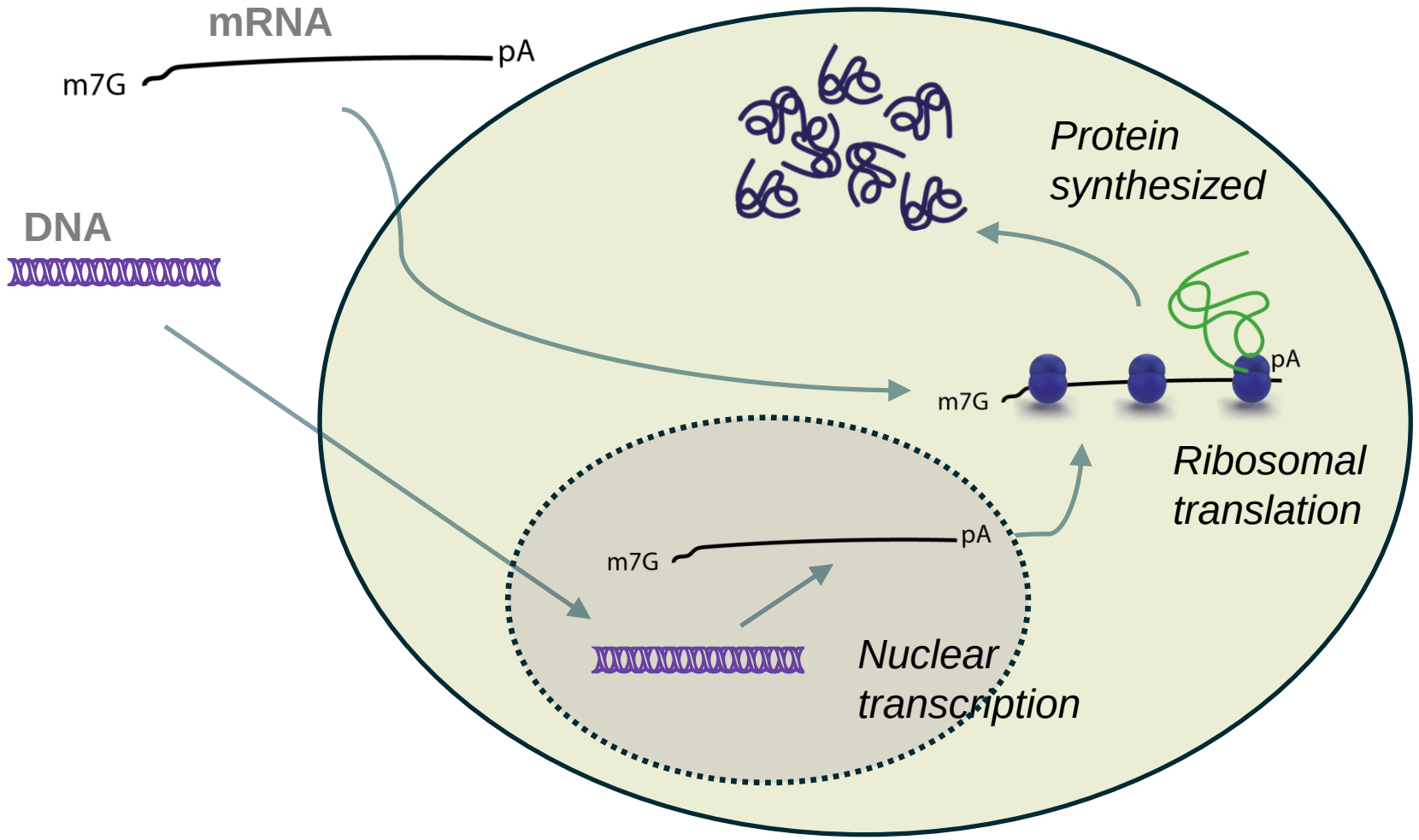
Gene therapy design

- Most forms of gene therapy involve delivery of DNA to patient cells
- Gene therapies contain synthetic genes (promoter + transgene + polyA)
- DNA sends an ‘mRNA’ message to the cell, instructing it to make its product

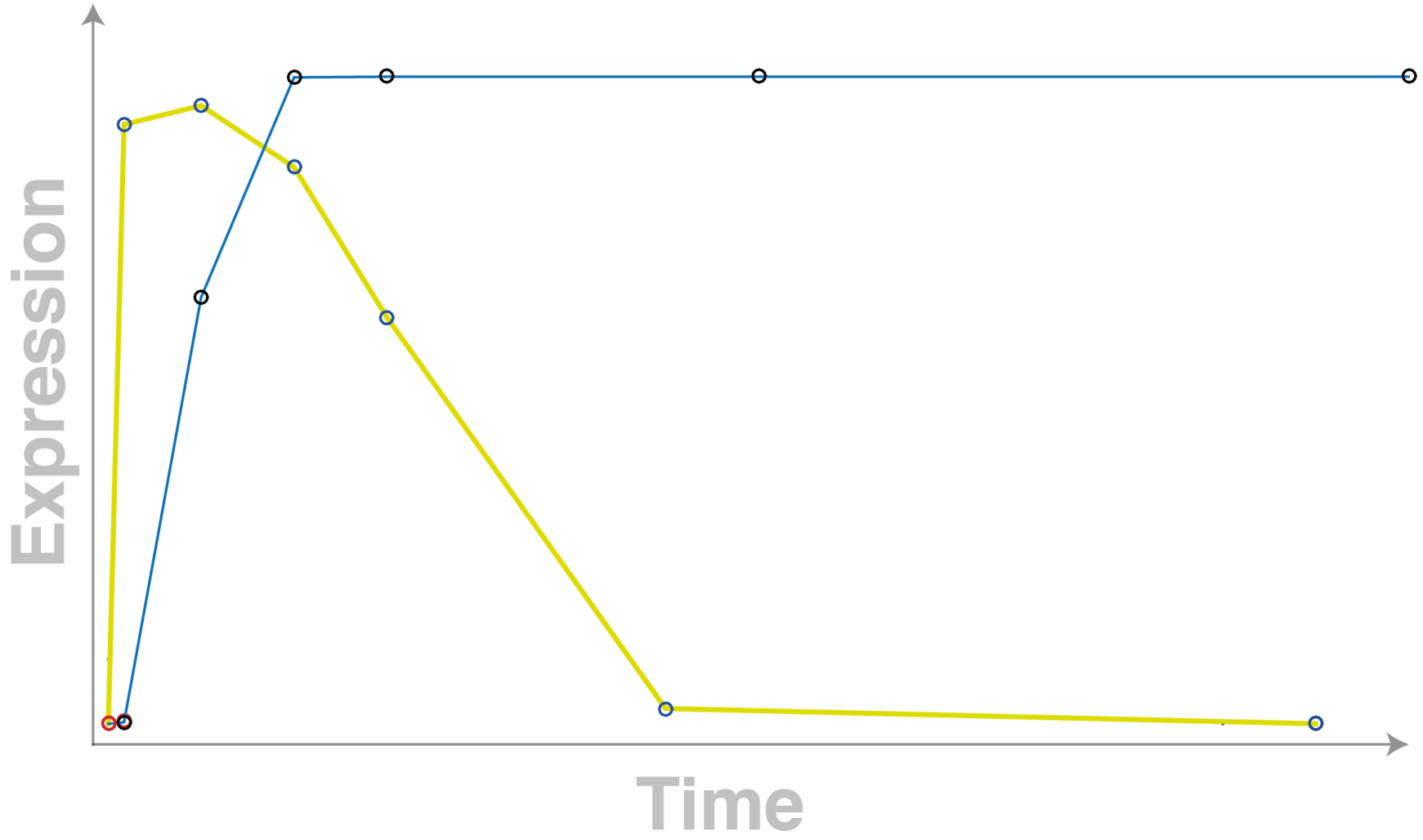


- mRNA can also be delivered directly to cells

DNA vs mRNA gene therapy



DNA vs mRNA gene therapy



DNA: Takes longer to switch on, but is active long-term

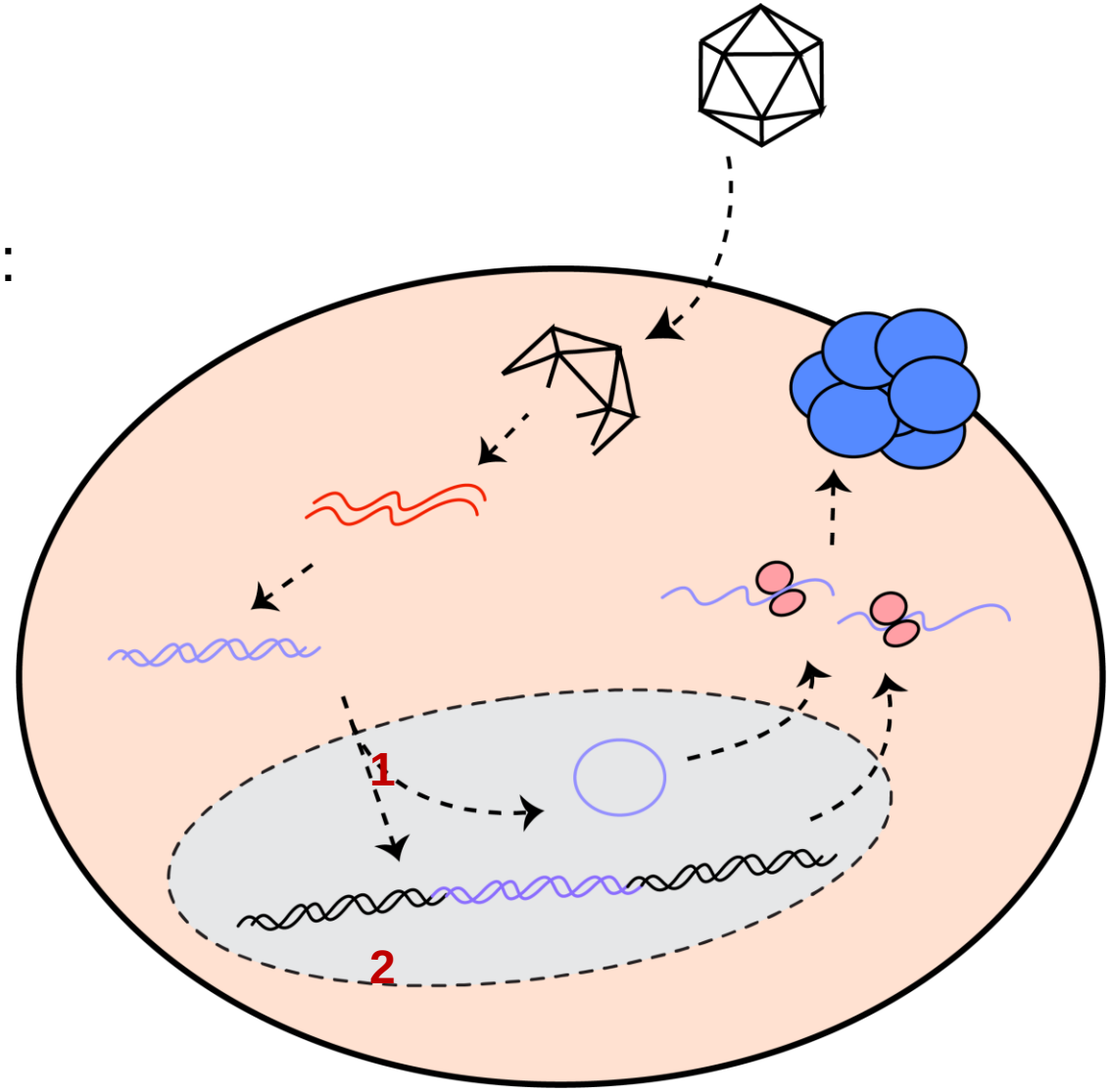
mRNA: Is immediate, but activity is transient

Integrated vs unintegrated DNA

However, gene therapy DNA can either be:

1. **Unintegrated** = transient/temporary

2. **Integrated** = stable/permanent



MSUD Research

- Discussions with doctor colleagues have highlighted MSUD as an area of interest for development of new treatments
- Identified 2 areas of focus:

1. Develop a gene therapy that can restore long-term BCAA metabolism in liver

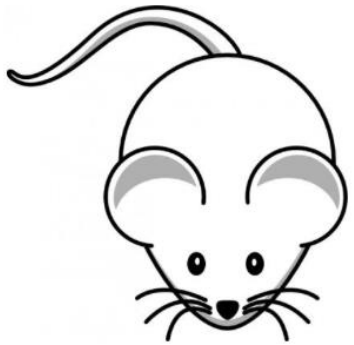
2. Identify a gene therapy that best protects the brain

MSUD Liver Gene Therapy Studies

- We are working with 2 types of gene therapy for liver
- **‘CCL’ Lentiviral vectors:** Integrate into the genome (stable)
- **AAV vectors:** Do not integrate very commonly (may be unstable in liver)
- Integration is an important aspect for gene therapy – if it integrates, it should be present lifelong
- If the gene therapy isn’t integrated, it can get ‘washed away’ over time as the liver grows and loses its effect
- However, gene therapies that integrate, like lentiviruses, have safety risks which we must approach with caution

MSUD Mouse Model

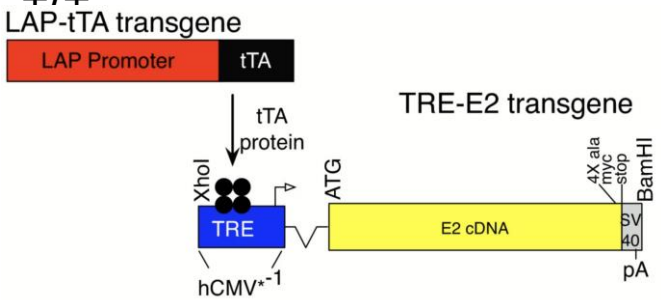
- MSUD mouse model established (Homanics et al, University of Pittsburgh)
- Mouse deficient in E2 (DBT) gene, with some residual activity



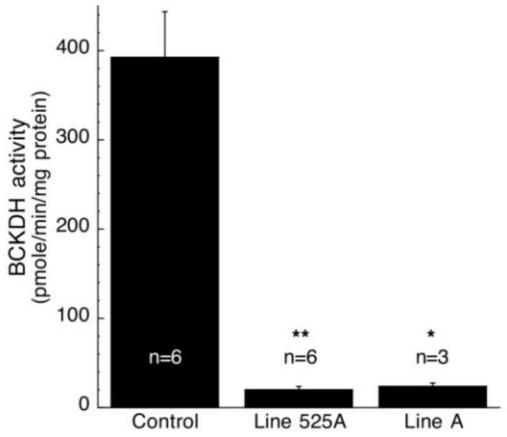
iMSUD

mDBT -/-

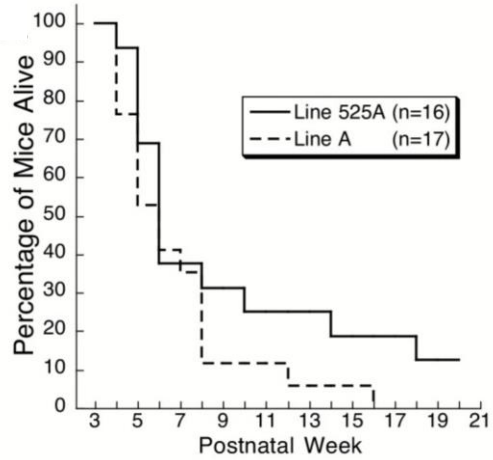
Liver-specific *hDBT* +/+



Homanics et al, BMC Med Genetics, 2006



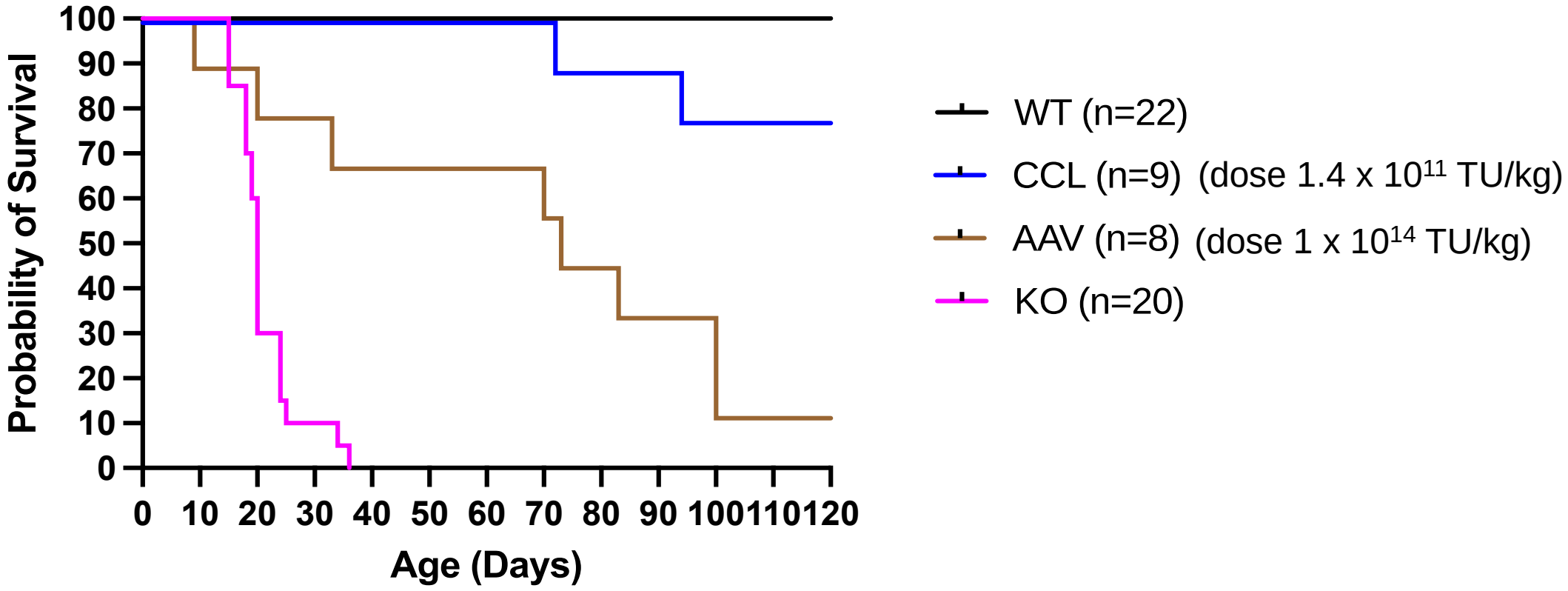
Reduced enzyme activity



Reduced survival

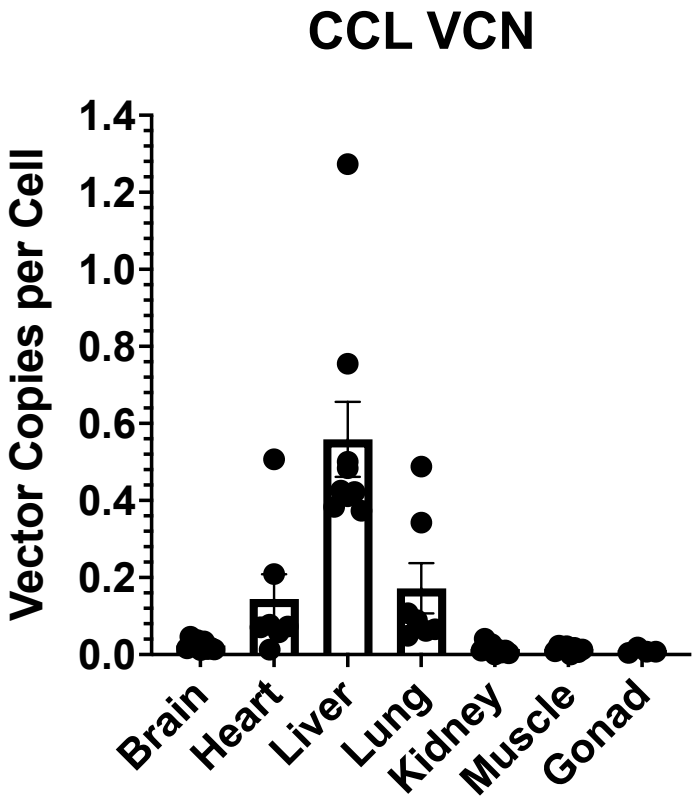
Mouse Survival Post-Treatment

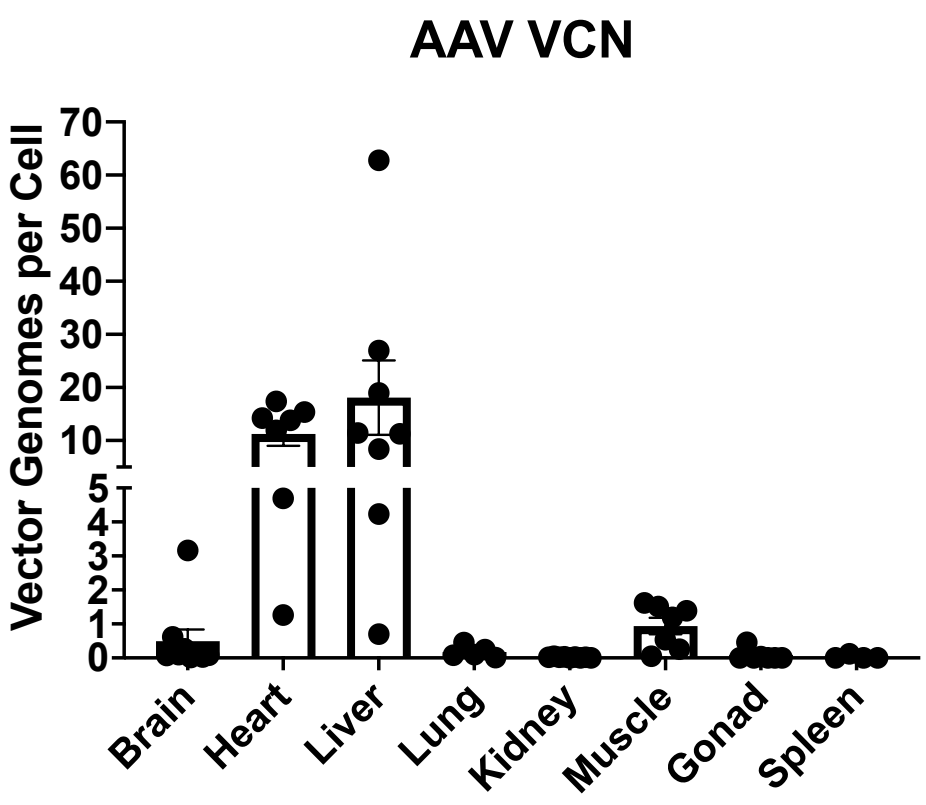
- Both treatments improved mouse survival – CCL (lentivirus) more so



Gene Therapy Delivery Rates

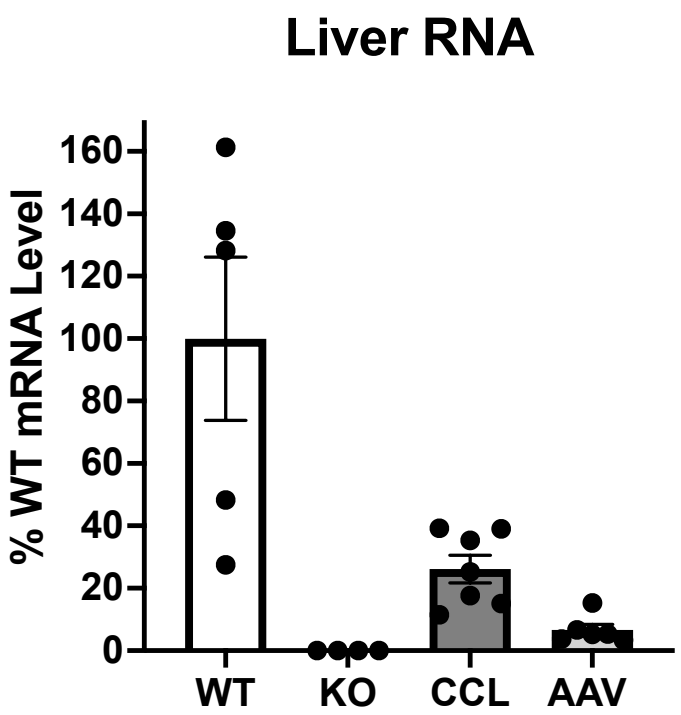
- Given differences in survival, wanted to check success of vector delivery
- Both vectors mainly delivered to liver but also other tissues (heart)

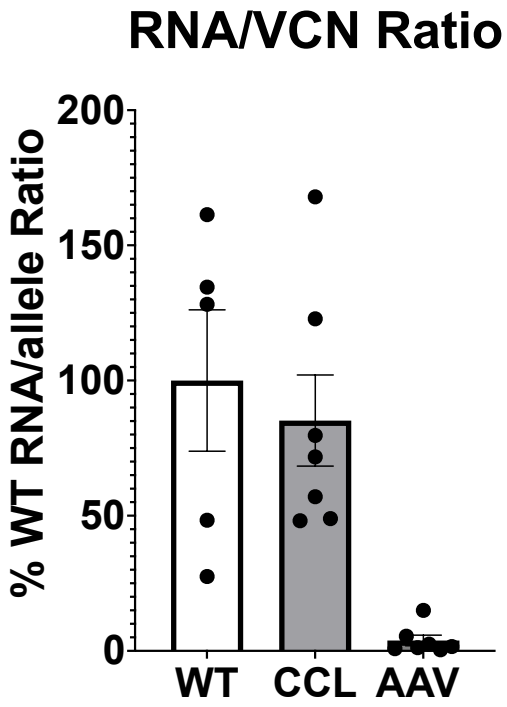




Vector Potency Comparison

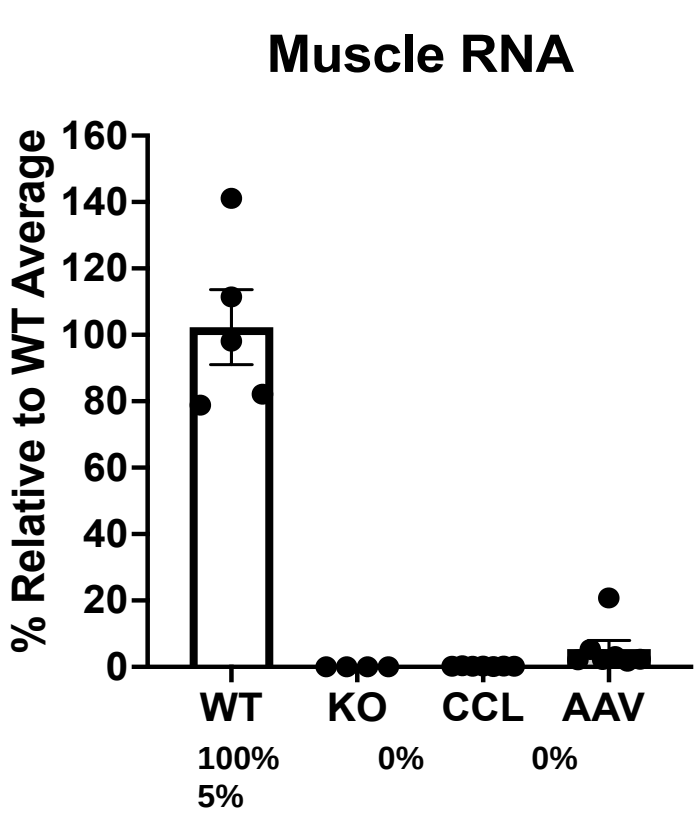
- mRNA is a marker of gene therapy activity
- mRNA levels showed different pattern - AAV less active in liver at later timepoints

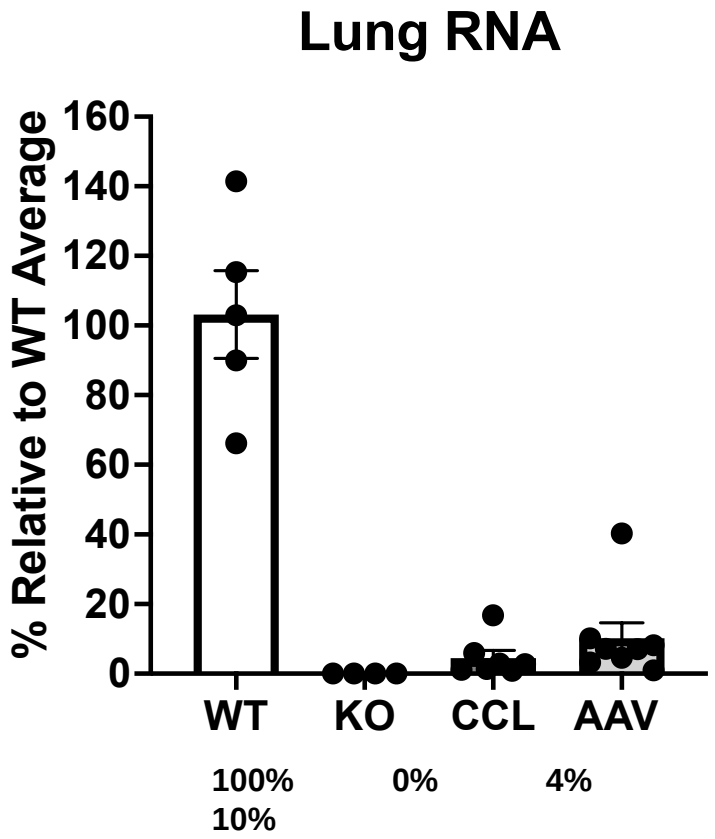


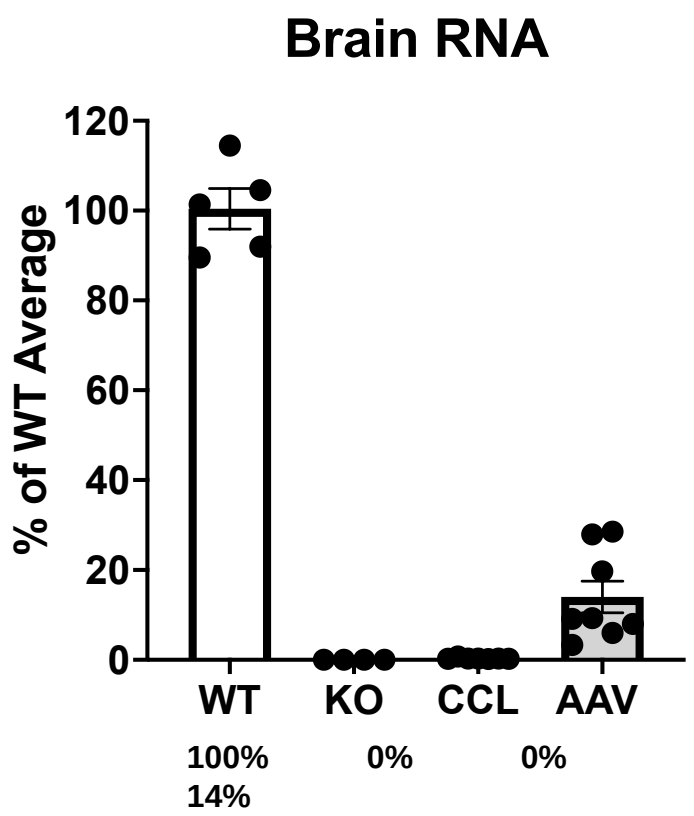


Vector Activity in Other Organs

- Investigated whether vectors were active in other organs
- Surprisingly, the AAV treatment was active in brain

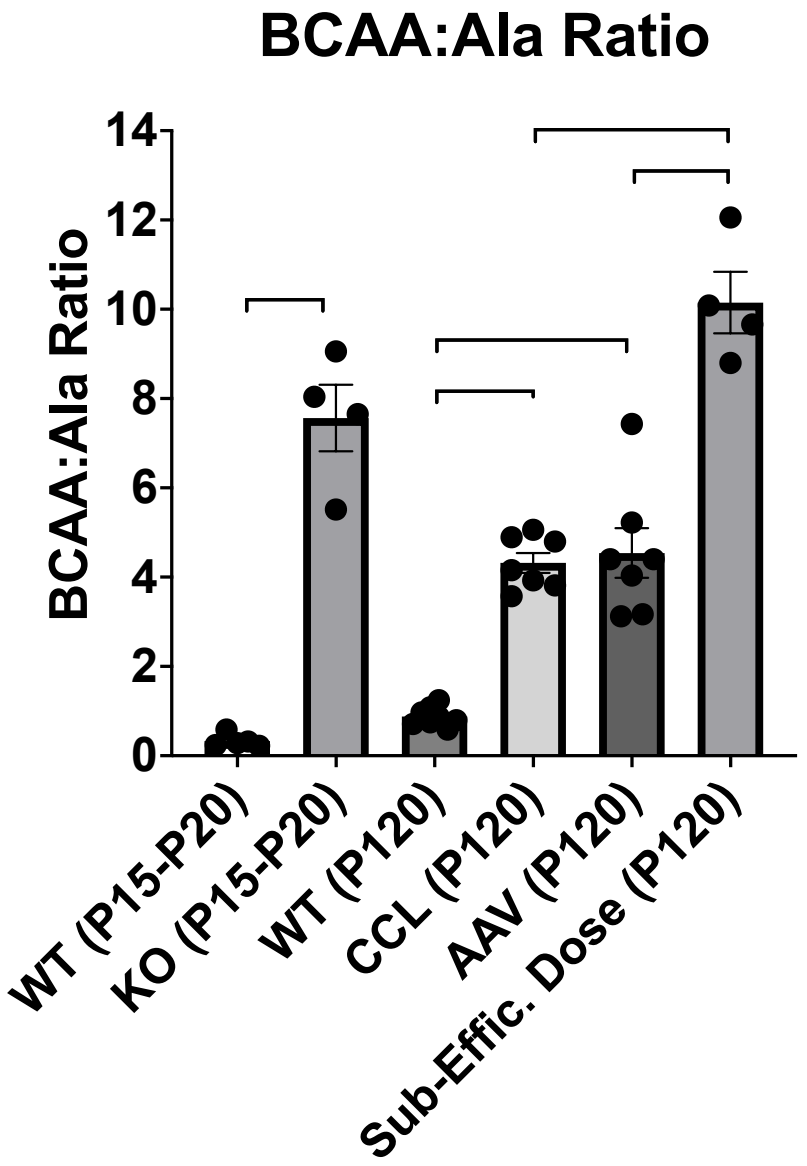






Plasma BCAA Levels

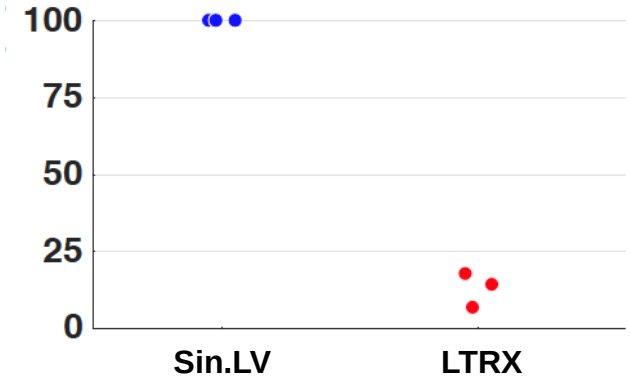
- BCAA levels were analysed
- As expected, these are elevated in MSUD mouse model
- Both gene therapies reduced BCAAs but not to low levels
- AAV benefit clearly not all coming from the liver - may be from brain expression



New technology: LTRx

- At UCL we have developed a new lentiviral gene therapy technology called LTRx

Patented: EP3058076, JP 2016539629, CN 105705645, US-2016-0230191

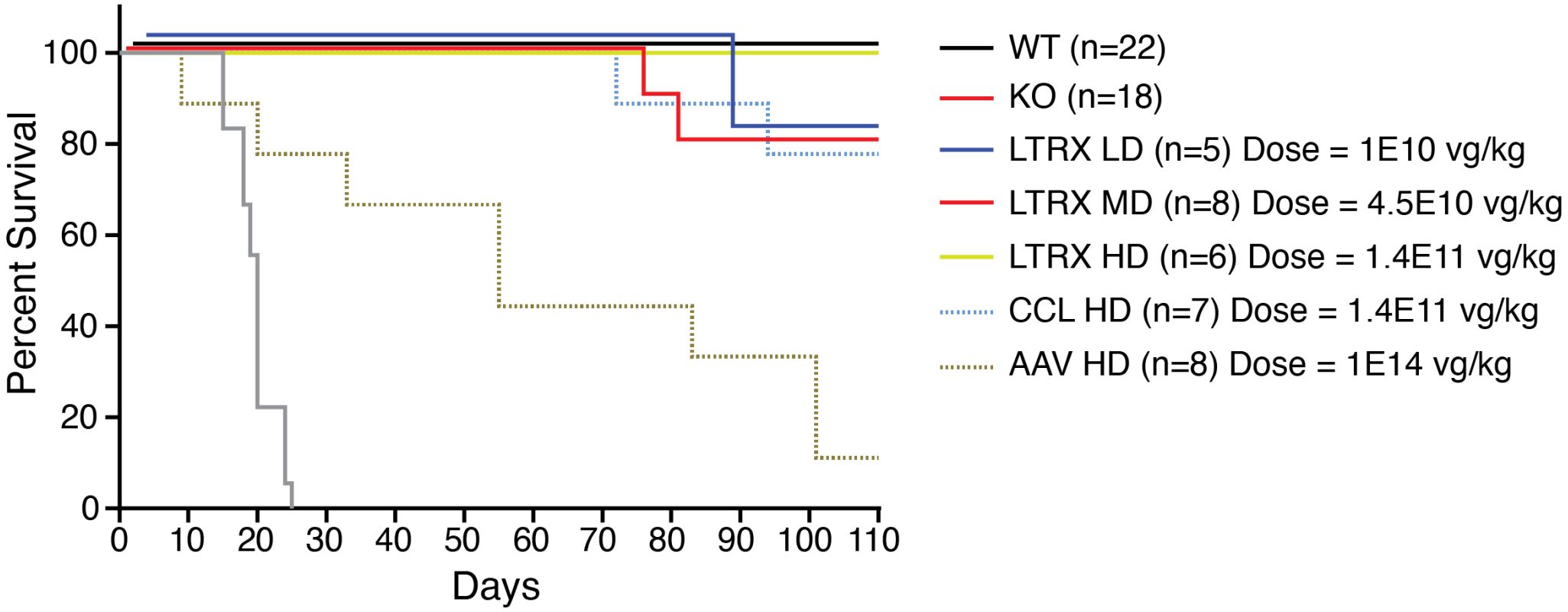


LTRX reduces ‘fusion transcripts’

- Designed to reduce side-effects associated with lentiviral gene therapy
- Previous studies found it works particularly well for liver gene therapy
- We’ve explored use of this technology for MSUD gene therapy

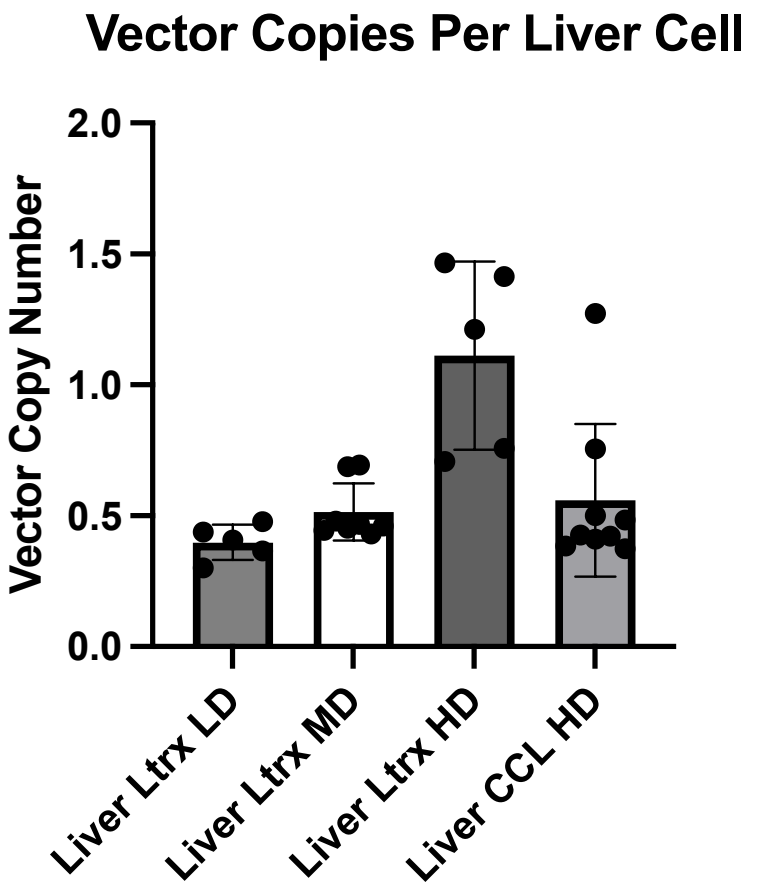
Mouse Survival Post-Treatment

- Mice received 3 different doses of LTRx
- 100% Survival at high dose



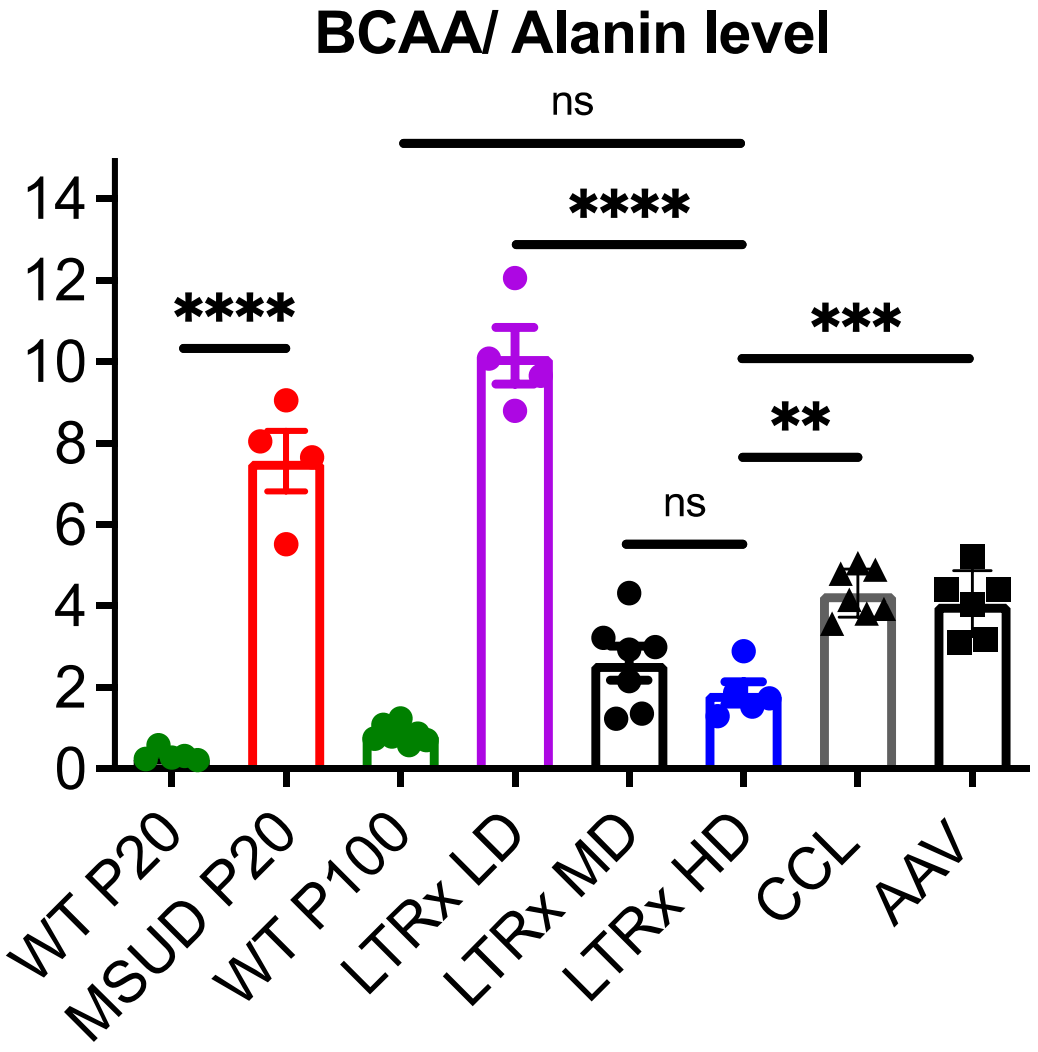
Efficiency of Vector Delivery to Liver

- LTRx appears to be more slightly more efficient at liver targeting
- The ‘CCL’ version is what most people are using in the field
- This might be a reason for greater effect on survival
- Further work being done right now to analyse the samples gained from these studies



Plasma BCAA Levels

- The LTRx high dose group appeared to have the best effect on BCAA levels
- Mid dose also worked
- Further work needed to understand the optimal dose range



Interim Summary

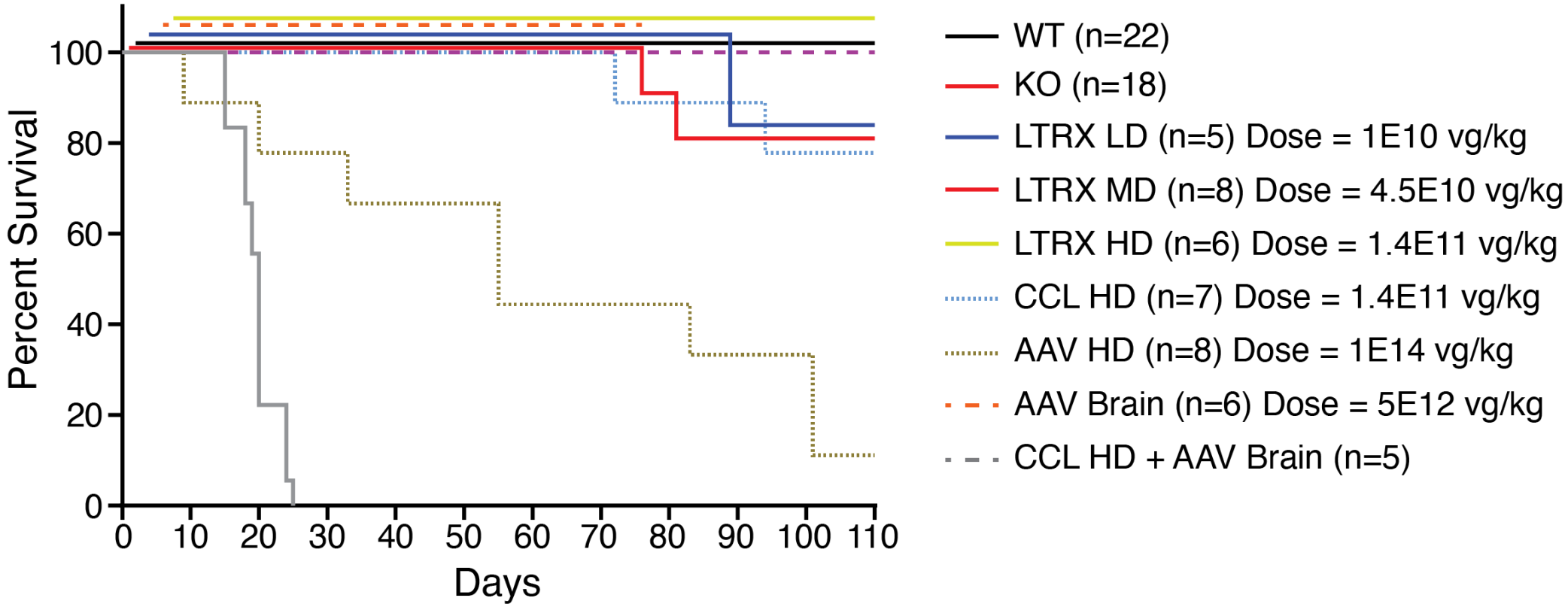
- LTRX technology appears to show promise for liver gene therapy
- Questions remain as to whether liver gene therapy alone would be sufficient
- We mentioned earlier that AAV gene therapy was found in the brain after delivery to the whole body
- How important is it to restore BCAA metabolism in the brain for MSUD?

MSUD Brain Gene Therapy Studies

- For brain gene therapy, we are only working with AAV
- AAV is much better than lentivirus for brain delivery
- Integration is much less important in the brain, as the brain grows in a different way, therefore gene therapy does not get ‘washed away’ like in the liver

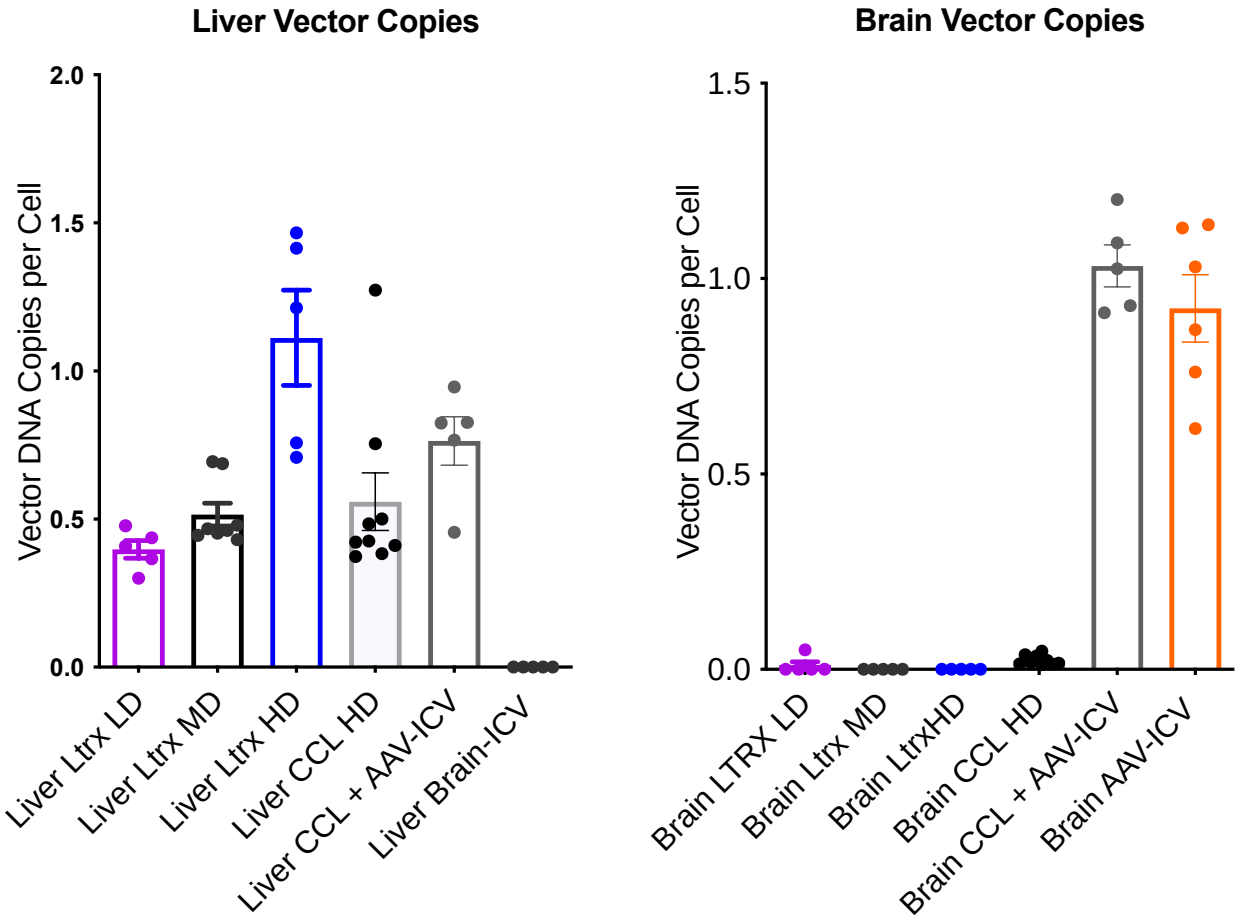
Mouse Survival Post-Treatment

- All mice survived after receiving brain-targeted gene therapy alone, or in combination with liver-targeted gene therapy



Efficiency of Vector Delivery

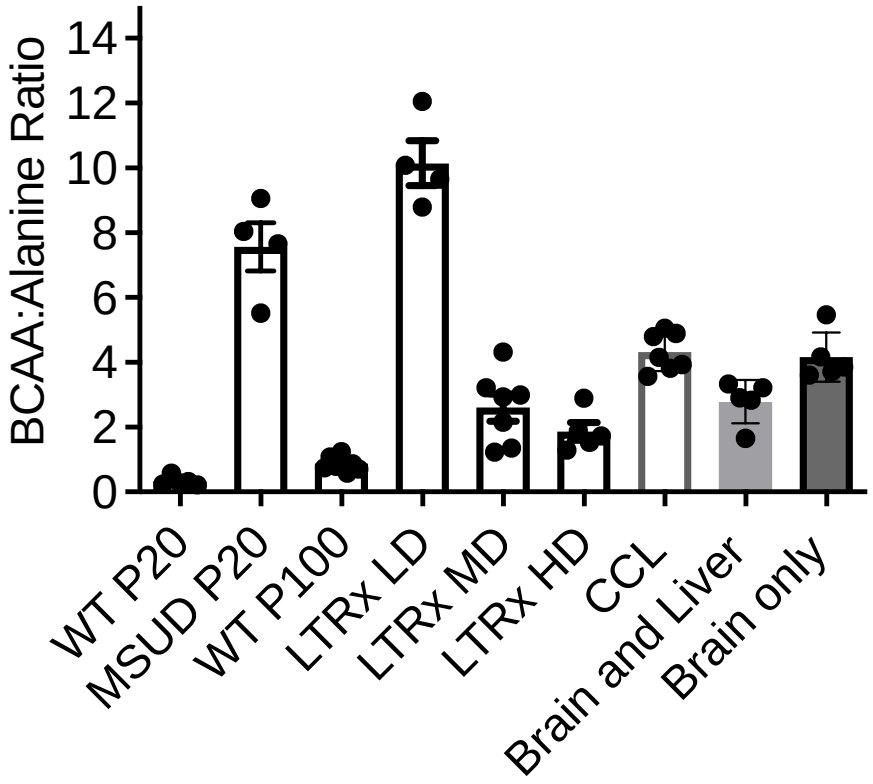
- Analysis confirmed that brain-targeted gene therapy was restricted to brain and the liver-targeted gene therapy was restricted to liver
- This helps us to confirm that the observations in our studies can be attributed to a mechanism of action



Effect on Plasma BCAA

- Surprisingly, brain gene therapy alone did reduce BCAA levels in the blood
- Combining liver and brain gene therapy did not reduce levels much further
- Further work will be focused on understanding which aspects of the disease are alleviated by brain gene therapy

Effect of Brain Gene Therapy on BCAA:Alanine Ratio



Final Summary

- Liver gene therapy with LTRx appears to produce best effect
- AAV produces higher rates of delivery to liver, but long-term potency is limited
- Delivering AAV to the brain alone is surprisingly effective, although it's unclear whether this is a direct effect on brain cells, or contributing to clearance of BCAAs in circulation

Current and Future Activities

- Additional experiments to identify the best doses to use for LTRx
- Demonstrate that it’s safe in additional laboratory studies
- Detailed analysis of brain samples from treated mice to understand how each type of gene therapy is impacting brain health
- Exploring further strategies to combine brain gene therapy with other means to control BCAAs in circulation

Thank you

UCL Division of Surgery and Interventional Science

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